PATHOGENICITY OF *EIMERIA TSUNODAII* FOR JAPANESE QUAILS (*COTURNIX COTURNIX JAPONICA*) AND SUSCEPTIBILITY OF THE COCCIDIDUM TO SOME DRUGS

Yoshiatsu TSUTSUMI  
*Daiichi Seiyaku Co., Ltd., Nihombashi-Edobashi, Tokyo, Japan*

Kiyoshi TSUNODA  
*National Institute of Animal Health, Kodaira, Tokyo, Japan*

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In their previous papers\(^{20-23}\), the authors reported the morphology, biological characteristics, localization, and host specificity of a coccidium which had infected the ceca of the Japanese quail, and gave the scientific name *Eimeria tsunodai* to this coccidium.

The present report deals with some observations on the pathogenicity of this coccidium, and its susceptibility to some coccidiostats.

**MATERIALS AND METHODS**

Oocysts for infection were prepared from pure cultures maintained from Japanese quails (*Coturnix coturnix japonica*) in strict isolation, using the method already described\(^{23}\).

Japanese quails, within a day after hatched in an incubator, were housed in complete protection from coccidial infection. Young birds 3, 15 or 30 days of age were used for experiments depending upon the purpose of examinations thereof. All of them were sacrificed until the 17th day following oral inoculation with oocysts and submitted to postmortem examination. Observation was made on pathological and clinical parameters (i.e., necropsy findings, including gross pathologic features of the ceca, the number of oocysts in the feces, and symptomatologic manifestations, including bloody diarrhea and mortality) to evaluate the clinical effectiveness of drugs.

Gross pathologic changes in the ceca were assessed according to the criteria adopted by TSUNODA in his report on *E. tenella* infection\(^{18}\).

For prophylactic and therapeutic trials, the 15-day-old quails were divided into 21 groups of 5 birds each.

Birds were supplied with feed containing specific doses of the test drug for three to seven consecutive days beginning with the day of infection. Initial doses were determined on the basis of usual therapeutic or prophylactic doses of the respective drug. Wherever sufficient clinical response failed to occur, dosage increase was undertaken accordingly.

**Drugs:** The drugs and doses (drug in feed \%) employed were as follows.

Amprolium (AM): 1-{4-Amino-2-N-prophyl-5-pyrimidinylmethyl}-2-picoliniumchloride hydrochloride .......................... 0.0125%, 0.05%, and 0.12%
Methylbenzoquate (MB): Methyl-7-benzyloxy-6-butyl-1,4-dihydro-4-oxoquinoline-3-carboxylate ........................................ 0.001%, 0.004%, and 0.04%
Clopidol (CP): 3,5-Dichloro-2,6-dimethyl-4-pyridinol ..................... 0.0125%
Sulfadimethoxine (SDM): 2,4-Dimethoxy-6-sulfanilamidopyrimidine ........
............................................................ 0.05%, 0.1%, and 0.2%
Sulfamonomethoxine (SMM): 4-Methoxy-6-sulfanilamidopyrimidine ........
............................................................ 0.05%, 0.1%, and 0.2%

RESULTS

1. Pathogenic features of infection with E. tsunodai.
Clinical symptoms: Groups of young quails ranging from 3 to 30 days of age were infected with $1.44 \times 10^2$ to $1.08 \times 10^6$ oocysts per bird. Clinical observation of these infected birds revealed that symptomatologic manifestations were practically the same in all the groups of birds having received oral infection with $10^3$ to $10^6$ oocysts per bird.
Watery diarrhea was observed on the 4th day following infection and bloodyecal contents were evident on the 5th to 8th days after infection. Blood stool was most prominent on the 5th and 6th days, and most deaths occurred during this period. The birds became entirely anorectic with their wings dangling and fell into a lethargic state. Eyelids and legs were often discolored because of anemia. The feathers around the cloaca were found in most cases soiled with bloody stool. These clinical symptoms

<table>
<thead>
<tr>
<th>No. of oocysts per quail</th>
<th>No. of quails</th>
<th>Age of quail</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>$1.44 \times 10^3$</td>
<td>10</td>
<td>20%</td>
</tr>
<tr>
<td>$1.44 \times 10^4$</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>$1.44 \times 10^5$</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>$1.08 \times 10^5$</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>$1.08 \times 10^6$</td>
<td>10</td>
<td>100</td>
</tr>
</tbody>
</table>

Chart 1. Body weight gain of quails suffering from cecal coccidiosis

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*Table 1. Mortality (%) in Japanese quails infected with different numbers of oocysts of Eimeria tsunodai*

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resembled to those of chickens infected with *E. tenella*.

Mortality: Table 1 summarizes the mortality of young quails infected with $1.44 \times 10^2$ to $1.08 \times 10^5$ sporulated oocysts per bird at 3, 15 or 30 days of age. In all the three age-groups about fifty per cent of the birds died following infection with $1.44 \times 10^4$ or more oocysts. There existed a tendency for the mortality rate to be higher with younger quails.

Weight gain: Chart 1 shows the effect of *E. tsunodai* infection on weight gain of five dosage of 5 young quails each having received $0, 1.44 \times 10^2, 1.44 \times 10^3, 1.44 \times 10^4, \text{and } 1.08 \times 10^5$ sporulated oocysts per bird respectively, by the oral route, at 3 days of age. As can be seen, there was no significant difference in bidaily pattern of weight gain between the uninfected control group and the group of birds given $1.44 \times 10^2$ oocysts. In all the other groups of infected birds it was evident that there was a relationship between the depression of body weight gain and the dosage level. Particularly, the quails of the group infected with $1.08 \times 10^5$ oocysts weighed only approximately 50% of the body weight of the birds of the uninfected control group on the 18th day after infection.

Gross pathologic findings: The lesion of coccidial infection was found to be localized in the ceca and rectum in all the birds examined, including heavily infected ones.

The cecum was found to be atrophic, having many bloody spots (petechiae) all over the mucosa, and was replete with a mixture of blood and cecated materials.

Predilectional site of parasites and histopathological findings: Japanese quails were experimentally infected with the pure strain of *Eimeria tsunodai*. From 2 to 5 infected birds were sacrificed at fixed intervals following inoculation with oocysts. The routine methods of histopathological observation were performed. Some of the specimens were stained by the PAS reaction for the purpose of demonstrating polysaccharides in the protozoa.

Microscopical examination of serial sections of the cecum and rectum revealed the presence of coccidia in various stages of development within the epithelial cells, lamina

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Drug</th>
<th>Concentration of drug in feed (%)</th>
<th>No. of infected oocysts ($\times 10^5$)</th>
<th>No. of birds studied</th>
<th>No. of dead birds</th>
<th>Cecal Lesion</th>
<th>Oocyst output</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amprolum</td>
<td>0.0125</td>
<td>1.4</td>
<td>1.4</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Amprolum</td>
<td>0.05</td>
<td>4.1</td>
<td>4.1</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Amprolum</td>
<td>0.12</td>
<td>1.4</td>
<td>1.4</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Methylbenzo-</td>
<td>0.001</td>
<td>1.4</td>
<td>1.4</td>
<td>5</td>
<td>2</td>
<td>1</td>
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<tr>
<td>5</td>
<td>Quate</td>
<td>0.004</td>
<td>4.1</td>
<td>4.1</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Clopidal</td>
<td>0.04</td>
<td>1.4</td>
<td>1.4</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Infected</td>
<td>0.125</td>
<td>1.4</td>
<td>1.4</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>Infected</td>
<td>4.1</td>
<td>5</td>
<td>4.1</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>Infected</td>
<td>1.4</td>
<td>5</td>
<td>1.4</td>
<td>5</td>
<td>2</td>
<td>4</td>
</tr>
</tbody>
</table>

* Daily oocyst counting was done for 9 days after infection. Its result was expressed as the number of oocysts per gram of feces. The figure given presents a maximum daily oocyst count.

** Oocysts were collected by the sugar-floatation method.
Table 3. Therapeutic effects of Sulfadimethoxine and Sulfamonomethoxine against E. tsunodai infection in Japanese Quails.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Drug</th>
<th>Dose (per cent in feed)</th>
<th>Duration of medication (days)</th>
<th>No. of infected oocysts ((10^4))*</th>
<th>No. of birds studied</th>
<th>No. of dead birds</th>
<th>Cecal lesion</th>
<th>Oocyst output</th>
</tr>
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<tbody>
<tr>
<td>10</td>
<td></td>
<td>0.2</td>
<td>5</td>
<td>4.1</td>
<td>5</td>
<td>0</td>
<td>5</td>
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<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>Sulfadimethoxine</td>
<td>0.05</td>
<td>5</td>
<td>4.1</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>0.1</td>
<td>3</td>
<td>4.1</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
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<tr>
<td>14</td>
<td>Sulfadimethoxine</td>
<td>0.05</td>
<td>3</td>
<td>4.1</td>
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<td>0</td>
<td>5</td>
<td>0</td>
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<td>2</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.2×10⁴</td>
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<tr>
<td>17</td>
<td>Infected control</td>
<td>0.2</td>
<td>3</td>
<td>4.1</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>0</td>
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<td>0</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>Sulfamonomethoxine</td>
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<td>5</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>Infected control</td>
<td>0.05</td>
<td>3</td>
<td>4.1</td>
<td>5</td>
<td>2</td>
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<td>4.1</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

* Daily oocyst counting was done for 9 days after infection. Its result was expressed as the number of oocysts per gram of feces. The figure given presents a maximum daily oocyst count.

** Oocysts were collected by the sugar-floatation method.

propria, lamina muscularis mucosae, and tunica muscularis. Young first-generation schizonts (PAS-negative) were observed within the epithelial cells, and matured second- or third-generation schizonts (PAS-positive) in a deeper portion of the lamina muscularis mucosae than the young schizonts. After second-generation schizonts had been formed, infected epithelial cells were destroyed considerably, and the lamina propria was infiltrated extensively with eosinophils, lymphocytes and plasma cells.

It was concluded that E. tsunodai was highly pathogenic for Japanese quails.

Prophylactic and therapeutic trials.

Table 2 summarizes the data concerning the response of Japanese quails to prophylaxis with some coccidiostats against E. tsunodai infection. Table 3 similarly shows a summary of the data regarding the response of the birds to treatment with sulfonamides against E. tsunodai infection.

Practically complete suppression of oocyst excretion was found to be accomplished in birds infected with E. tsunodai by administration with AM (group No. 3), MB (No. 6) and CP (No. 7) at a concentration of 0.12, 0.04, and 0.125 per cent, respectively in feed for 7 consecutive days. Equally, administration with 0.05–0.2% of SD and SM was effective on experimental coccidiosis caused by E. tsunodai when it was started on the day of infection and continued for 3 consecutive days. None of the drugs herein used were found to have side effects when administered at the above-specified dosage levels.

DISCUSSION

Mazurkiewicz made a report on E. tenella found in Japanese quails kept under laboratory conditions. Ohuchi et al. have detected a disease characterized by hemor-
Pathogenicity of *Eimeria tsunoda* and Susceptibility of Coccidium

rhage and swelling in the cecum from among Japanese quails reared in a field ranch, and observed coccidial oocysts in the feces.

*E. tsunoda* coincides with the coccidium described in the above-cited two reports in terms of the parasitic location, mortality, morbidity, and symptoms. It is, therefore, suspected that these reports might have been related to collective outbreaks of *E. tsunoda* infection.

*E. tsunoda* is, however, evidently different from those reported by Nakayama¹¹-¹³, Tsunoda¹⁰, Bhatia¹ and Norton¹⁵ in endogenous development and pathogenic feature. This would mean that several varieties of *Eimeria* might be detected from Japanese quails.

It was also found that *E. tsunoda* was much more pathogenic than *E. bateri* and *E. uzura*. Therefore, it is important further to study the prophylactic and therapeutic means against the disease caused by *E. tsunoda* in animal industry.

Nakayama¹¹-¹³ used sulfaquinaxaline throughout his studies with some success, but failed to carry out sufficiently effective control on coccidia. He proposed the usefulness of colinycin in conjunction with the sulfaquinaxaline therapy. Mazurkiewicz et al.⁸ reported a failure in their attempts to treat the disease with “DOT-soluble-Byk” (medication: 9 days) and to prevent it with “Furazol W” (medication: a few days). They had used Japanese quails suffering from acute cecal coccidiosis as laboratory animals. Mazurkiewicz and his associates consequently concluded that the disinfection and sterilization of cages were the most advisable.

Ouchi and his coworkers¹⁶ reported outbreaks of coccidiosis in the colonies of quails maintained on a quailfarm. Therapeutic and prophylactic trials were conducted against this disease with some antiprotozoal and sulphonamide agents. For the therapeutic purpose, a sulphonamide preparation (a mixture of sulfamethazine, sulfathiazole and sulfadimethoxine) was added to the feed given to the affected birds for 6 consecutive days. As a result of the medication, a significant reduction in mortality was accomplished, although a sharp increase in death rate followed the subsequent discontinuation of the medication. As prophylactic feed-additives, 0.006 to 0.12% of AM, and 0.05% of furazolidon were used with little success against coccidiosis in chickens. Eventually, it was emphasized to use sufficient doses for prophylactic and therapeutic measures against coccidiosis in Japanese quails.

It was reported by Tsutsui²³ that mature schizonts of *E. tsunoda* demonstrated the PAS-positive reaction. There is an author report purporting to have demonstrated a marked efficacy of antithiamine drugs on coccidiosis of chickens due to infection with PAS-positive coccidia. The usefulness of AM in the control of coccidiosis of chickens has been described by many investigators (Cuckler⁵, Long⁷, and Katae et al.⁸).

When the results obtained from the present study in Japanese quails were compared with those from the previous studies in chickens, it was indicated that ten to twenty times the dose of AM effective for chickens was necessary to accomplish the same magnitude of anticoccidial effectiveness in quails infected with *E. tsunoda*.

Studies were carried out by Bowie²⁹, McFadyen⁹, and Spencer¹⁷ on the clinical evaluation of quinolone derivatives as anticoccidials for chickens. In the present study, treatment with doses of MB forty times the effective dose reported for chickens, however, failed to suppress the oocyst excretion completely in quails infected with *E. tsunoda*.

Though prescribed only at one dosage level in the present study, CP was proved to have the same effect as reported in the previous papers against chicken coccidiosis.

Since the first report by Levine⁶, numerous papers have been published on sulphonamide therapy in chicken coccidiosis. Clinical trials were carried out by Tsunoda¹⁸,
Ito\textsuperscript{4} and Mitrovic et al.\textsuperscript{10} to treat avian coccidiosis with sulfadimethoxine and sulfamonochromoxine. It appears that these drugs are most frequently used. In comparison with the previously reported results in chickens, the data obtained from the whole studies herein described stress the remarkably high efficacy of these drugs against cecal coccidiosis of Japanese quails.

**SUMMARY**

1) Several groups of Japanese quails were infected with *Eimeria tsunodai* at the age of 3, 15, and 30 days to assess clinically the pathogenicity of newly isolated coccidial strains. These birds displayed a fairly high mortality rate and marked symptomatic manifestations, thereby indicating the high pathogenicity of the strains.

2) Clinical assessment was made on ammonium (AM), methylbenzoquate (MB), clopidol (CP), sulfadimethoxine (SDM), and sulfamonochromoxine (SMM) on Japanese quails against infection with *E. tsunodai*. As a result, CP, SDM, and SMM had a significantly high anticoccidial effect against *E. tsunodai*.

**REFERENCES**

日本ウズラ（*Coturnix coturnix japonica*）から分離した
*Eimeria tsunodai* の病原性と薬剤感受性について

提 可 厚
第一製薬株式会社，開発部

角 田 清
農林省家畜衛生試験場

（昭和46年8月3日受付）

前報で, *Eimeria tsunodai* の形態，生物学的性状，発育環，宿主特異性について報告した。今回は病原性を観察するため，3, 15, 30日令の日本ウズラに1.44×10^5から1.08×10^6の*E. tsunodai*の成熟オーシストを感染させた。

*E. tsunodai* のオーシストを10^3個以上感染させた場合，体重の増加が著しく妨げられ，10^4個以上の感染群では，発症率が50%以上であった。血便と貧血を主徴とした症状の，*E. tenella* 寄生によるニワトリの症状に酷似していた。また，*E. tsunodai* 人工感染日本ウズラを用いて，Ampicillin (AM), methylbenzoquate (MB), Clopidol (CP), Sulfadimethoxine (SDM) および Sulfamonomethoxine (SMM) に対する薬剤感受性を検討した。

試験の結果，AM, 0.12%; MB, 0.04%; CP, 0.125%を飼料に添加し，7日間連続した群，SDMおよびSMMを0.05%3日間投与した群で，*E. tsunodai*の増殖が阻止された。鶏の盲腸コクシジウム（*E. tenella*）と*E. tsunodai*とは，薬剤感受性の点で大きな差異を認めた。

EXPLANATION of PLATES

PLATE I

Fig. 1. Sick Japanese quail (left) on the 8th day after experimental infection with *Eimeria tsunodai*.

Fig. 2. Dilated hemorrhagic ceca.

PLATE II

Fig. 3. Second-generation schizonts in ceca 3 days after infection.

Fig. 4. PAS-positive zygotes in ceca 5 days after infection.